ABSORPTION ENHANCERS

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INTRODUCTION

There is enormous literature on the use of absorption enhancers. Here, the most important absorption enhancers for topical, transdermal and mucosal drug delivery are reviewed.

TOPICAL AND TRANSDERMAL

It is generally accepted that the bioavailability of most topically applied drugs remains low. Various methods to increase this bioavailability have been used. One of the approaches is the use of absorption enhancers, and over the years, there has been a great interest in new chemical absorption enhancers. An absorption enhancer should be pharmacologically inert, nontoxic, have a rapid and reversible onset of action, be chemically and physically compatible with other formulation compounds, and be cosmetically acceptable (1). Of course not all absorption enhancers possess all of these characteristics, and a benefit–to–risk evaluation will determine the choice of a molecule as an absorption enhancer. The range of absorption enhancers that has been researched is large. Thus, overview of the most researched compounds is presented.

Alcohols and Polyols

Some solvents are able to remove lipids from the stratum corneum, and several topical preparations (e.g., gels) and transdermal reservoir systems contain high concentrations of ethanol capable of modifying the lipid content of the skin (2). Solvents, such as ethanol, but also others such as propylene glycol, *N*-methylpyrrolidone, and Transcutol™, might also increase the drug flux through the skin by increasing the solubility of the permeant in the skin. It has also been suggested that the activity of propylene glycol results from solvatation of α-keratin within the stratum corneum, hereby promoting permeation by reducing drug-tissue binding.

Amines and Amides

Some excipients might intercalate into the structure of lipids of the skin and disrupt the ordered packing making so the structure more fluid and influencing positively the diffusion coefficient. Azone® and its analogues have been widely studied in that respect, and it has been shown that the hydrogen bonding between the polar head group in Azone® probably interacts with the skin ceramides (3). Godwin et al. (4) compared the penetration-enhancing ability of a wide range of pyrrolidone compounds, including those with different chain lengths and functional groups. Using hydrocortisone as a model drug, these authors suggested that *N*-dodecyl-2-pyrrolidone and its acetate analogue were the two most effective penetration enhancers using in vitro hairless mouse skin model. Several studies dealed also with the mechanism of action of Azone® and its analogues. Compounds with short alkyl chains, such as *N*-methylpyrrolidone, seemed to have no effect on the phase transition temperature and probably work through its action of solvency rather than through a structural change of the skin barrier function. Using multilamilar DDPE liposomes, Hadgraft et al. (3) showed that their phase transition temperature was lowered by the Azone and its analogues in the same rank order as their enhancing abilities. This indicates that the modifier activity might be related to the fluidising effect on the lipid lamellae.

Studies involving the structure activity relationship of several groups of enhancers showed that the presence of a cyclic structure in the molecule plays an important role in the activity determination of the enhancers. In addition, the greatest barrier disruption activity was recorded for compounds with long alkyl chains between C_8–C_{16} (5). Unfortunately, these molecules show also irritating potentials (6). Recently, Hadgraft (7) described some new molecules with similar structures but with low irritance potential.

Urea promotes transdermal permeation by facilitating hydration of the stratum corneum and the formation of hydrophilic channels (8).

Fatty Acids

The perturbation of the intercellular lipid bilayers in the stratum corneum seems to be the most important reason

for the enhancing activity of fatty acids such as oleic acid. Oleic acid has been described to decrease the phase transition temperatures of the skin lipids with a resultant increase in motational freedom—or fluidity—of these lipids (9).

Terpenes

Mono- and sesquiterpenes are known to increase percutaneous resorption by increasing the diffusion in the stratum corneum and/or disruption of the intercellular lipid barrier (10, 11). It has been shown that there is a major difference between different types of terpenes: e.g., it was shown that *d*-limonene did not disrupt the intercellular bilayers, whereas 1-8-cineole seemed lipid disruptive at physiological temperatures (12).

Menthol also has been described as a potential penetration enhancer due to its preferential distribution into the intercellular spaces of the stratum corneum and its possible reversible disruption of the intercellular lipid domain (13).

Esters

A typical example of an ester acting as a penetration enhancer is isopropyl myristate. Isopropyl myristate might show a double action: influence on the partition between vehicles and skin by solubilization and disruption of lipid packing, thus increasing the lipid fluidity (14, 15).

Sulfoxides

Dimethylsulfoxide (DMSO) has been found to be a potent enhancer, but unfortunately high concentrations which produce irreversible skin damage, erythema, and wheals, are required to obtain a desired effect. Recently, novel molecules were produced by modifying DMSO, by replacing the oxygen atom with a nitrogen atom that was substituted with an arylsulfonyl, aroyl, or aryl group. The *S*, *S*,-dimethyl-*N*-(4-bromobenzoyl)iminosulfurane produced the highest activity. But these compounds require more activity and toxicity studies, especially in less permeable models such as the human skin (16).

Cyclodextrins

Cyclodextrins can form inclusion compounds with an increase in solubility of lipophilic compounds, but they seemed less effective alone than in combination with fatty acids and propyleneglycol (17).

Surface Active Agents

The effect of surface active agents on the skin barrier function depends on the agent's chemical structure. In general, anionic surfactants tend to be more effective than cationic ones, whereas nonionic surfactants are considerably less effective. Most anionic surfactants can induce swelling of the stratum corneum, as well as uncoiling and stretching of α-keratin helices, thereby opening up the protein controlled polar pathways (18).

The impact of anionic surfactants is a function of the alkyl chain length of the molecule. A maximum was observed for surfactants having a linear alkyl chain of 12 carbon atoms (e.g., sodium lauryl sulphate). Unfortunately, anionic surfactants are reported to be irritative. Nonionic surfactants might increase the membrane fluidity of the intercellular regions of the stratum corneum (e.g., Brij®) and may extract lipid components and additionally, though of minor importance, they might interfere with keratin filaments and create a disorder within the corneocytes (19). It should be emphasized that surfactant form micelles which, if used above their CMC, might negatively influence the drug bioavailability.

Other Enhancers

Other potential penetration enhancers have also been described, such as *N*-acetylprolineesters (20) and glyceryl monocaprylate/caprate (21).

It should be emphasized that the activity of any enhancer should be evaluated in terms of function of the vehicle used and that the selection of the combination enhancer-vehicle is a function of the final therapeutic objectives.

(TRANS)MUCOSAL PERMEATION ENHANCERS

At all mucosal sites, the coadministration of absorption enhancers is normally necessary to achieve therapeutic relevant plasma levels of (large) hydrophilic molecules such as peptides or proteins. Table 1 gives an overview of the most used and researched absorption enhancers and their possible mechanism of action.

Next, an overview is given of the most used transmucosal drug delivery routes and the use of permeation enhancers in each of them.

Table 1 Most used and researched mucosal permeation enhancers

Type	Examples	Mechanism of action
Synthetic surfactants	Laureth-9 sodium lauryl sulphate polysorbate 20 and 80 PEG-8 laurate sorbitan laurate glyceryl monolaurate saponins (e.g., Quillaja saponins)	membrane interaction extraction of membrane proteins and lipids solubilization of peptides
Bile salts	sodium deoxycholate sodium glycocholate sodium fusidate sodium taurodihydrofusidate	denaturation of proteins decrease of mucus viscosity decrease of peptidase activity solubilization of peptides formation of reversed micelles
Fatty acids and derivatives	oleic acid caprylic acid lauric acid palmitoylcarnitine	fosfolipid acylchain disruption
Chelators	Na_2EDTA citric acid salicylates	Ca^{2+} complexation (influencing tight junctions)
Inclusion complexes	cyclodextrins and derivatives	increasing peptide stability increasing solubility enzyme inhibition
Other agents	Azone®	lipid structure disruption

Oral and Buccal Mucosa

There are clear differences between the oral mucosal membrane and other epithelial membranes of the intestine, nasal cavity and rectum. The oral mucosal membranes are less keratinized than the skin membranes and show a more loosely packed intercellular lipid domain. In terms of function of the absorption enhancement through the oral mucosal membrane, it can be said that it occurs principally through the lipid-filled intercellular spaces. One could suggest that the mechanism of increasing lipid fluidity of intercellular lipids, as indicated previously for the skin, should also apply for the oral mucosal membranes (22). As has been reported in the case of skin, other mechanisms can be applied here. For example, sodium deoxycholate appeared to denature and extract proteins from rabbit buccal mucosa and affected membrane lipids and inhibited proteases.

There are only a limited number of studies comparing the systematic changes in the structure of enhancers and their influence on the oral mucosal membranes. For example, for insulin absorption in rats, it was shown that sodium glycocholate, laureth-9, sodium laurate, and sodium lauryl sulphate were approximately equipotent. Several nonionic surfactants having a C_{12} hydrophobic tail were much less effective (23, 24).

A study related to the buccal bioavailability of testosterone indicated the absorption enhancing effect of hydroxypropyl-β-cyclodextrine with a relative bioavailability of 165% versus the administration without absorption enhancers. This effect was probably due to an increased solubility of testosterone, although cyclodextrins might also extract lipids from the intercellular matrix (25). In the same study, sodium tauro-24,25-dihydrofusidate and sodium deoxycholate did not show any enhancing properties.

Nasal Mucosa

Many papers have been published on the use and efficacy of absorption enhancers for nasal peptide and protein

delivery. The enhancing effect of bile salt seemed dependent on its lipophilicity: The bioavailability of gentamicin increased with increasing lipophilicity of trihydroxy bile salts (cholate > glycocholate > taurocholate), and the enhancement of nasal insulin bioavailability followed the rank order of deoxycholate, chenodeoxycholate, and cholate. However, most studies reported severe damage of bile salts to the mucosa. Deoxycholate had the most ciliotoxic effect, whereas taurocholate had the least ciliotoxic effect (26). In the case of dihydrofusidates, a dose-dependent increase in bioavailability was reported for peptides such as insulin.

A number of dihydrofusidate derivatives have been synthesized in order to evaluate the stucture-enhancement relationship. Acidic derivatives achieved a higher enhancement than basic derivatives, but the safety of dihydrofusidates remains a contradictory issue and some structural damage to the mucosa has been reported (27).

In the past years, much research has concentrated on the use of cyclodextrins to enhance bioavailability of peptides and proteins especially because of their mild and reversible effect on the nasal mucociliary clearance (28).

Among the cyclodextrins, the use of DMβCD was shown to have the highest effect on the transnasal bioavailability of insulin in rats. Several studies reported on their concentration-dependent effect. Besides for peptides, the methylated β-cyclodextrins have shown to be useful in nasal delivery of lipophilic drugs. The toxicological profile of dimethyl β-cyclodextrins and of randomly methylated β-cyclodextrins appeared excellent. Attention should be paid, if possible, on bioavailability differences between animal and human models.

Vaginal Mucosa

Laureth-9, lysophosphatidylcholine and palmitylcarnitine chloride were found to be highly effective absorption enhancers, but all induced epithelial damage (29). Insulin was also administered to ovariectomized rats, and the coadministration of sodium taurodihydrofusidate, laureth-9, lysophosphatidylcholine, and -glycerol significantly increased hypoglycemia. Lysophosphatidylglycerol showed only minor damage of the vaginal epithelium, in contrast to the other absorption enhancers used (30). The combination of lysophosphatidylcholine and starch microspheres showed promising insulin bioavailability results (31).

Deoxycholate and quillajasaponins were reported to have a positive effect on the vaginal absorption of calcitonin (32).

Rectal Mucosa

Due to a combination of poor membrane permeability and metabolism at the site of absorption, rectal bioavailability of peptide and proteins is low. As in other mucosal bioavailability testing, insulin is the most studied polypeptide with respect to rectal absorption.

Sodium salicylate and 5-methoxysalicylate increased the absorption of insulin (33). Sodium glycocholate was more effective than sodium taurocholate but less effective than sodium-deoxycholate and PE-9-laurylether in enhancing rectal insulin absorption in rabbits (34). The role of disodium EDTA in the enhancement of rectal drug absorption, along with the damaging effects on the rectal mucosa, has been described for several drugs (35, 36).

Bile salts were also used for the enhancement of drug absorption, but several studies indicated severe damage due to their use in rectal drug delivery (37). Sodium tauro-24, 25-dihydrofusidate (STDHF) had a positive effect on the availability of cefoxitin, vasopressine, and insulin in rats (38).

The possible use of mixed micelles (e.g., made of unsaturated fatty acids and monoglycerides) has been shown for the enhanced rectal absorption of several compounds, including α and β interferon and insulin.

Pulmonary Absorption Enhancers

Only a few studies are available related to the effect of known absorption enhancers on the pulmonary absorption of poorly absorbable drugs, including peptides and proteins.

It was reported that oleic acid, oleyl alcohol, and Span 85 can increase the transfer rate of disodium fluorescein in isolated rat lungs (39). Pulmonary insulin absorption was reported to be increased in the presence of glycocholate and Span 85 (40). Fluorescein isothiocyanate, insuline, and a calcitonin analogue were better absorbed when coadministered with *n*-lauryl β-D-maltopyranoside, sodium glycocholate, and linoleic acid mixed micelles (41). The same authors, however, reported on the toxicity of *n*-lauryl β-D-maltopyranoride. A large number of questions are still remaining, such as why sodium caprate enhances the bioavailability of phenol red and isothiocyanate-labeled dextrans but not of insulin and a calcitonin analogue.

Hydroxypropyl-β-cyclodextrin and especially dimethyl-β-cyclodextrin have been shown to enhance the pulmonary bioavailability of insulin in rats, and indications were found of a relatively low acute mucotoxicity (42).

Table 2 Some of the commonly used intestinal absorption enhancers

Bile salts	Sodium cholate, sodium deoxycholate
Nonionic surfactants	Polysorbates and polyoxyethylene alkyl esters and ethers
Ionic surfactants	Sodium lauryl sulphate and dioctyl sulfosuccinate
Fatty acids	Sodium caprate, oleic acid
Glycerides	Medium-chain glycerides, phospholipids
Acyl carnitines	Palmitoylcarnitine
Chelating agents	EDTA
Swellable polymers	Polycarbophil and chitosan

Intestinal Absorption Enhancers

The optimization of oral bioavailability is of common interest because low bioavailability is often the cause of variable and poorly controlled clinical and toxic effects. This is of major importance for polar molecules such as peptides and proteins (43).

Table 2 reviews the most commonly described compounds to enhance intestinal absorption and indicates some examples (44).

It should be emphasized that absorption enhancers might act selectively on some parts of the GI tract, and this fact implicates that the formulation will play a major role in the optimal delivery of drug and absorption enhancers.

Bile salts have proven to act very differently on the intestinal absorption of drugs. In some cases, the drug absorption was reduced due to micelle formation, whereas in other cases, the absorption was enhanced due to intestinal membrane disruption caused by the solubilization of phospholipids or by Ca^{2+} complexation (45–47).

Nonionic and anionic surfactants haven been shown to be able to enhance the intestinal absorption of drugs. Some studies have shown that in the area of nonionic surfactants ethers were more effective than esters, but this phenomenon was not always confirmed. There is an indication that the surfactants cause membrane damage, which can be correlated with their enhancement activity (48). It has been shown that some tensioactive agents might influence tight junction permeability (49).

Fatty Acids

Fatty acids increase intestinal absorption via their influence on the paracellular and transcellular transport route. Most interesting results were obtained with lauric acid, palmitic acid, capylic acid, and oleic acid or their salts. Cytotoxic effects of fatty acids are concentration dependent long-chain unsaturated fatty acids especially can cause epithelial cell damage (50–52).

Glycerides

Medium-chain glycerides (mainly C_8–C_{10}) are known to increase the intestinal absorption of poorly permeable drugs, mono- and diglycerides, especially, improve bioavailability, and it is believed that mainly transcellular permeation is increased.

It should be emphasized that the formulation plays an important role in the effect of these glycerides (emulsification, enteric coating, etc.) The main advantage of these products is their general acceptance for use in oral drug administration (53, 54).

Finally, it should be noted that during the last decade both weakly crosslinked poly(acrylic acid) derivatives and chitosan derivatives were described as safe penetration enhancers for hydrophilic compounds especially as they can trigger mechanisms of tight junction opening of mucosal tissues and did not show acute toxicity. Poly(acrylic acid) derivatives were shown to have excellent mucoadhesive properties and can inhibit the activity of gut enzymes, such as trypsin, chymotrypsin, and carboxypepsidases (55, 56). Chitosan salts and N_1-trimethylchitosan chloride revealed to be potential absorption enhancers for nasal absorption of calcitonin and insulin and for the intestinal absorption of buserilin (57).

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